The Effects Development has on the Redistribution of Tubulin in Sea Urchin Embryos

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Introduction

Studying sea urchin (*Lytechinus pictus*) embryos at different stages of development through the use of immunofluorescent stainting and epifluorescent microscopy helps find where tubulin are located in cells. The developmental study of sea urchins is important because the tubulin movement in sea urchins is similar to the tubulin movement in humans during the early stages of development. In this study epifluorescent microscopy is used so that tubulin can be located in the cell when a blue epifluorescent light (FITC) is introduced to the embryos that have been stained and blocked and placed onto a coverslip. When the light is introduced to the coverslips they emit a green color that represents the areas where the tubulin are located in the cell. In this study, my hypothesis is that the tubulin does not remain uniform in developing cells, and if the embryo cell presents a bright green color, then there is a high concentration of tubulin in that specific area, and if the embryo cell appears to be a dim green color, then amount of tubulin within the area are less concentrated. The disassembling and reassembling of the microtubules will be displayed by the strength of the green light that the immunofluorescents attach to. This hypothesis was tested in a different way by Patricia J. Harris, Elise L. Clason, and Kevin R. Prier, and they discovered that after sometime during the gastrula stage of development the total amount of polymerized tubulin (similar patterns with total tubulin) appeared to reach a peak, with all tubulin fluorescence present in certain strong areas and very little background fluorescence in the cytoplasm (Harris, 1989). This is an important hypothesis to research because the first clear phenotypic cellular differentiation event that takes place during embryonic development is cileogenesis which is the formation of cilia, which are affected by tubulin and microtubules. Abnormal cilia can cause ciliopathies that can cause blindness, hearing issues, and smelling issues. Understanding the concentrated locations of tubulin during certain points of development help people understand what causes ciliogenesis.

Tubulin is a common protein that is a principle component of microtubules. The two most common tubulin are α tubulin and β tubulin (the α subunit is where antibodies bind), which both make up microtubules. Microtubules have an important role with different forms of cellular movement during cell division. They are located in the cytoplasm, in the axoneme of flagella and cilia, and in basal bodies which are structures located at the base of the cilium. Microtubules help with the transportation of cellular organelles, vesicles, and are responsible for the placement of cellular compartments. These important trafficking organelles are labile because of the developing cells constant polymerizing and depolymerizing. This occurs because the microtubule skeleton needs to be remodeled after each mitosis, so it is constantly disassembling and then reassembling. The location of the microtubules varies according to the cell type and the stage at which the cell is at during mitosis. The shrinking and growing of microtubules allows for them to expand into different regions of the cytoplasm and for the cell to move as it divides. The blastula is one cell layer thick, and during gastrulation cells become thinner as they expand (Gilbert, 2006). Before cellular division occurs, bipolar spindles are created with the rearrangement of the microtubules, so that the chromosomes can be separated and eventually split into two cells. This process occurs as the embryo continues to develop (Amos, 2005). During gastrulation, the archenteron extends dramatically, and cells in the archenteron rearrange themselves by migrating over one another and by flattening themselves. Tubulin help with filopodial extensions of secondary mesenchyme to help pull up the archenteron and elongate it (Gilbert, 2006). The more cells in a specific area of the cell, the more tubulin are present, and the brighter the image will be in that area.

Method

I followed the Morris 2009 Immunofluorescent Staining of Sea Urchin Embryos lab instructions with minor adjustments. For step 9, the MeOH was not taken out of the 1.5 ml tube with the mid gastrulas. For step 12, instead of using PBS-T, block buffer was used. For step 13, instead of using the polylysine, protamine sulfate was used in order to

make the glass surface of the coverslips cationic. Additional block buffer was added in step 16, and for step 19 the embryos were blocked over night. Step 29 took one hour and five minutes to complete, and instead of filling the three wells on the well plate with PBS-T they were filled with block buffer. In step 39, the embryos were incubated in the secondary antibody for two hours. In step 49, the embryos were incubated in the second primary antibody for one hour and forty minutes (Morris, 2009). A Nikon E400 epifluorescent microscope was used with standard Hoechst, FITC, and Rhodamine fluorescent filter sets, using Spot Advanced Software on a Spot Insight camera from Diagnostic Instruments. A 40x plan fluor objective was used for all images.

Results

For this experiment a cell in the four-stage embryo and a blastula cell of a sea urchin were observed in order to show that tubulin does not remain uniform in developing cells and where they are located. The four-celled embryo's cells appeared much larger than the blastula's cells, which allowed for it to be much easier to see the definition of the cell than the blastula cells. A green color is shown in the areas where tubulin are located in the cells, and this can be seen in Figures 1 and 2.

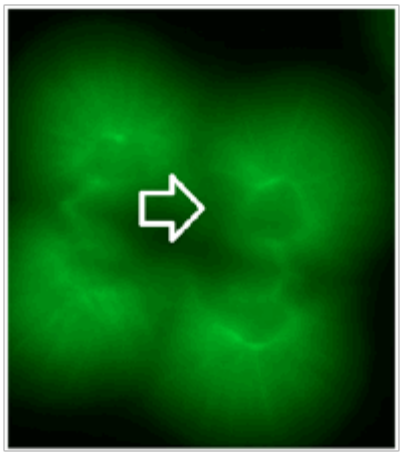

Figure 1: This cropped image is of a sea urchin four-cell embryo during mitosis after interphase. The location of the tubulin can be determined by where the green color is located in the cell. The cell that the white arrow points to is the cell whose brightness is observed and measured in Figure 3. Notice the somewhat consistent brightness of each cell within the four-cell embryo.

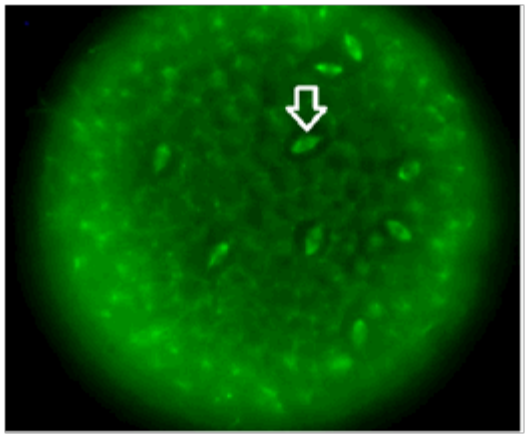

Figure 2: This cropped image is of a sea urchin blastula, and it displays the total amount of tubulin in the developing blastula. The tubulin are located where there is green color. The cell that the white arrow points to is the cell whose

brightness is observed and measured in Figure 3. Notice the bright green fluorescencts in the individual cells located within the blastula that allows for some of the cells mitotic stages to be determined.

The brightness in the cell of the four-cell embryo varies less than the brightness in the blastula cell. An individual cell in the four-celled embryo is the dimmest around its edges, but then becomes brighter as it gets closer to the center of the cell, and then it becomes dimmer in the middle, (but not as dim as it does around the edges of the cell). The blastula cell has a similar pattern where it starts off much dimmer (than the cells in the four-celled embryo) around the cell's edges and then quickly becomes very bright closer into the middle of the cell, but in the middle of the cell the green color minutely dims down. The brightness in the blastula cell varies drastically and immediately, where as the cell in the four-cell embryo changes in brightness, but is more consistent in color and is not as drastic. The two variations in brightness in the cells are compared in figure 3. Being able to compare the variations of brightness of the two cell's at different developmental stages allows for there to be a comparison between the location and strength in polymerization of tubulin.

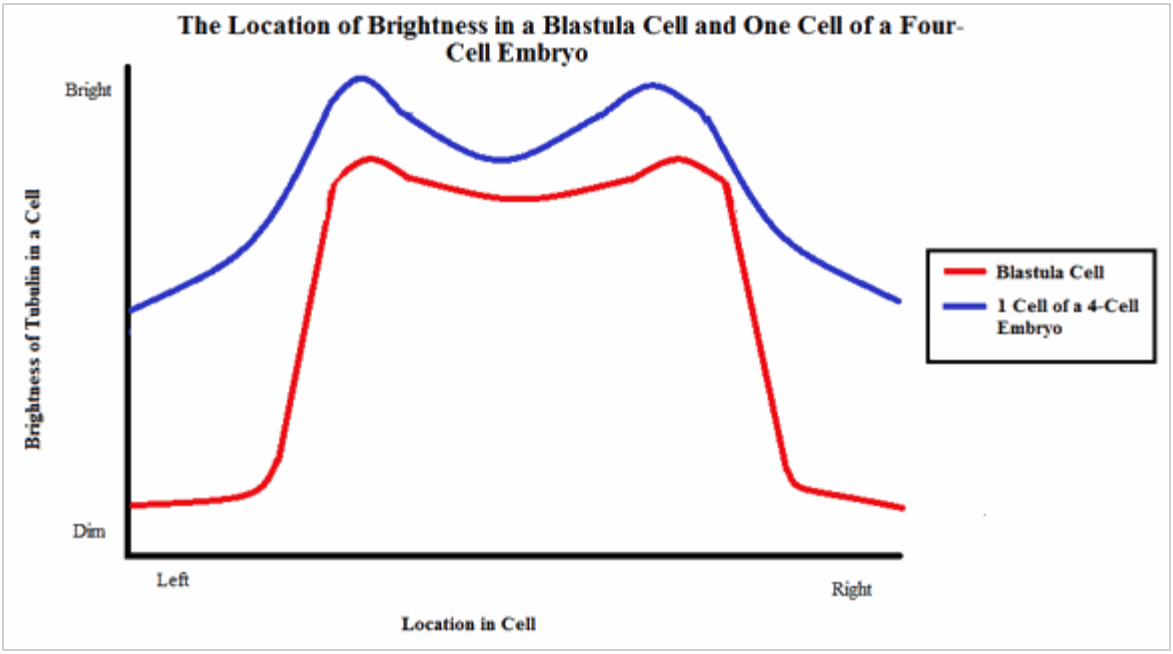

Figure 3: This graph displays semi-quantified information about the amount of brightness in a blastula cell as well as in one cell of a four-celled embryo from left to right of each cell. The brightness in each cell reflects on where the tubulin are located and whether they are polymerized or diffused. The dimmer the cell appears, the more diffused it's tubulin is, and the brighter the cell is, the more concentrated the tubulin is. Notice how both the blastula cell and the one cell of a four-celled embryo have similar patterns in their brightness, but the 1 cell from a four-celled embryo appears to be brighter than the blastula cell.

Conclusion

After completing the experiment my hypothesis, that tubulin do not remain uniform in developing cells, and if the embryo presents a bright green color, then there is a high concentration of mitotic cells with continuously changing tubulin in that specific area, and if the embryo appears to be a dim green color in the cell, then amount of cells within the area are less concentrated, was supposed. It can be concluded that tubulin located in cells at early stages of development such as the cells in four-cell embryos are more polymerized than the tubulin in cells at the blastula stage.

The tubulin and cytoplasm in the four-cell embryos is much brighter than they are in the blastula stage and this is because the cells in young embryos like the four-celled embryo are more reliant upon polymerized tubulin to help the cells undergo mitosis and transportation of vesicles than they would be in later stages. Another possible reason why the cells appeared much dimmer in later stages (blastula) of early development is because as the embryos develop and become a blastula there is a thinning of cells that are located away from where the cell's archenteron will be because the cell is getting ready for invagination (Gilbert, 2006). The spreading out of cells makes more room for the tubulin to expand which then makes it appear as if the tubulin is less concentrated than they appear in a larger four-cell embryo. When the tubulin appear less concentrated their green fluorescent appears dimmer, and when the tubulin is more concentrated within the cell it appears much brighter. The blastula cell and the cell from the four-cell embryo have similar brightness patterns because their tubulin are located towards the center of the cell. The reason why the brightness of the cells decreases in the very center of the cell in because that is where the DNA is located, and on the overlay images there is a bright blue color suggesting that that is where they are placed.

There were no sources of error in my experiment, but in order to refine my experiment and make my results more reputable, next time it would be beneficial to use Photo Shop to find out how bright the cells were by measuring the brightness of the pixels from the two different cell images. Having this information would allow for me to have numbers to create a reputable graph as opposed to making assumptions as to how bright each cell is at certain areas. For a future experiment, I think that it would be interesting to observe where the tubulin are located in a gastrulated cell and compare it to a blastula cell to gain further knowledge of the patterns of the developmental process of sea urchins.

Works Cited

Amos, Linda A., (2005) "Tubulin and Microtubules." *Encyclopedia of Life Sciences* 10 ed.

Collaborated with Professor Morris's Developmental Biology class

Harris, Patricia J., Clason, Elise L., and Prier, Kevin R., "Tubulin Polymerization in Unfertilized Sea-Urchin Eggs Induced by Elevated Temperature." *The Journal of Cell Science 93.* (1989): 9-17. Print

Morris, Robert., (2009) "Immunofluorescent Staining of Sea Urchin Embryos, MeOH Fixation (For Classroom Use, Adherent Embryo Technique)." Pg.1- 5.

S.F. Gilbert. Developmental Biology. 8th ed. Sinauer Assoc. Sunderland, MA. 2006